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ORIGINAL ARTICLE

S100A Expression and *Interleukin-10* Polymorphisms Are Associated with Ulcerative Colitis and Diarrhea Predominant Irritable Bowel Syndrome

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Abstract
Background Both ulcerative colitis (UC) and diarrhea-predominant irritable bowel syndrome (IBS-D) are associated with alterations in enteric serotonin (5-HT) signaling.
Aims The purpose of this study was to compare the rectal and sigmoid colonic mucosal expression of S100A proteins and functional polymorphisms of the 5-HT transporter (5HTT) and interleukin-10 genes in patients with IBS-D or UC with healthy controls.
Methods mRNA expression of S100 proteins was measured in sigmoid and rectal biopsies and in rectal epithelium isolated by laser-captured microdissection. Leucocyte DNA was analyzed by PCR-based reaction fragment length polymorphisms and direct sequencing. Clinical symptoms were assessed by the self-rating depression scale and by the gastrointestinal symptom rating scale.
Results Fifty patients with IBS-D, 56 with UC and 50 healthy controls were studied. Colonic mucosal expression of *S100A8* and *S100A9* in UC was significantly higher than in IBS or controls and correlated with the UC disease activity index ($r = 0.65$, $p < 0.001$). S100A10 expression in the rectal epithelium of the IBS patients was significantly higher (0.643 vs. 0.402, $p = 0.01$) than in controls and correlated with the SDS scores ($r = 0.41$, $p = 0.002$). The frequency of *IL10-819* CC genotype was significantly higher in IBS-D (10.7 vs. 0 %, $p = 0.047$) and UC (16 vs. 0 %, $p = 0.007$) than that in controls.
Conclusion Overexpression of S100A10 in the rectum may play a role in IBS as it is involved in modulating 5-HT1B receptors. The *IL10-819* CC is a candidate genotype for both IBS and UC in Japanese.

Keywords S100A10 · Interleukin-10 · Serotonin transporter

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Introduction

Irritable bowel syndrome (IBS) is a common, chronic, gastrointestinal (GI) disorder with variable manifestations. The Rome III criteria are currently used to categorize IBS patients on the basis of the predominant symptoms such as diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), a mixture of both diarrhea and constipation (IBS-M), and a group of un-subtyped IBS [1]. Mucosal inflammation is not a predominant feature of IBS, whereas ulcerative colitis (UC) is a chronic inflammatory colon disease. Despite the marked differences both IBS and UC often present with similar symptoms of abdominal pain, bloating and diarrhea, and in both there is evidence of disordered enteric serotoninergic signaling.

Serotonin [5-hydroxytryptamine (5HT)] is an important neurotransmitter and paracrine signaling molecule in both the central nervous system and the gut and is involved in the regulation of GI motility, sensation, and secretion, as well as nociception, mood, and anxiety [2, 3]. The predominant site of 5HT synthesis, storage, and release in the

intestine is the enterochromaffin (EC) cells present in the intestinal mucosa. Rapid reuptake of 5HT into presynaptic nerve terminals or epithelial cells of the GI mucosa by the 5HT transporter (5HTT) is thought to play a critical role in regulating the intensity and duration of serotonergic signaling [4–6]. Evidence for disordered enteric serotoninergic signaling in IBS and in UC includes an increase in the number of EC cells and of the mucosal content, release and uptake of 5-HT [7–13]. However, how 5-HT may be involved in the pathophysiology of UC or IBS remains unclear.

Polymorphisms in the 5HT transporter are thought to affect the regulation of translation and expression of 5HTT in the gut [14–17]. The 5HTT gene-linked polymorphic region (5HTT-LPR) consists of a 44 base pair (bp) insertion/deletion in the 5′ flanking promoter region of the gene creating long (*l*) and short (*s*) allelic variants, respectively [18, 19]. Another polymorphic site in the 5HTT gene is in intron 2 and is related to a variable number of tandem repeats (VNTR) consisting of 17 bp repeats [19]. VNTR and 5HTT-LPR polymorphisms have been investigated for possible associations with fibromyalgia, anxiety, and depression, all of which are common comorbid conditions in patients with IBS [20–24]. Moreover, imbalances of pro- and anti-inflammatory cytokines and polymorphisms in cytokine genes have been reported in IBS. For example, several polymorphisms have been identified in the promoter region of *IL-10* gene [e.g., −1082 A/G (rs1800896), −829 C/T (rs1800871), and −592 A/C (rs1800872)] [25] and these single nucleotide polymorphisms (SNPs) are closely related to the expression of IL-10 [26]. However, SNPs of IL-10 or 5HTT-LPR have not been consistently observed in IBS and inflammatory bowel disease (IBD) and their role thus remains unresolved.

S100 proteins comprise a family of more than 20 calcium-binding proteins which are characterized by their tissue-specific expression patterns. S100A8 (also named calgranulin A; myeloid-related protein 8, MRP8) and S100A9 (calgranulin B; MRP14) are present in granulocytes, monocytes and early differentiation stages of macrophages [27]. Expression of these proteins can also be induced in epithelial cells under inflammatory conditions [28, 29]. S100A8/A9 and calprotectin (the hetero complex formed by non-covalent association of S100A8 and S100A9) are potentially markers of gut inflammation and also thought to possibly be involved in the IBD pathogenesis [30–33]. To date most studies have focused on fecal levels of calprotectin as a marker of inflammation rather than in relation to its expression in the mucosa [31, 34–36].

S100A10 (also known as p11) co-localizes with 5HT1B receptors. Stimulation of S100A10 results in relaxation of the gastric fundus and a delayed stomach emptying in humans [37]. S100A10 expression is reduced in brain tissue from depressed patients and is increased in rodent brains by antidepressants [38]. The role of S100A10 in IBS is unclear. Camilleri et al. [39] previously reported overexpression of S100A10 in the sigmoid and rectum of IBS patients, but reports concerning the role of A100A10 in IBS or UC have not yet appeared. The aim of this study was to study mRNA expression of S100A and the functional polymorphisms of 5HTT and IL-10 genes in the patients with IBS-D compared to UC and healthy controls.

Methods

This was a case–control study of patients with UC or IBS-D and age- and sex-matched healthy controls. Subjects were enrolled for the study between August 2009 and January 2013. The study was approved by Kawasaki Medical School Ethical Committee, and written informed consent was obtained from each subject.

Subjects

UC

Requirements for subjects with UC included a previous tissue diagnosis and a careful review of their medical history, endoscopy reports, and pathology reports. The disease activities of UC patients were assessed according to the Sutherland DAI score which is the sum score of the following four parameters (each scoring between 0 and 3, making 12 the highest score): stool frequency, rectal bleeding, mucosa appearance, and physician's global assessment [40].

IBS

The diagnosis of IBS was clinical and based on the Rome III criteria [1]. IBS-D was defined as >25 % of stools being loose (mushy) or watery. Subjects with other subtypes of IBS were excluded. Subjects with a self-reported organic GI disorder (peptic ulcer disease, IBD, malignancy, gallbladder disorder, pancreatitis, or liver disease) and previous surgery of the GI tract were excluded. IBS patients underwent colonoscopy to rule out other organic colon diseases.

Healthy Subjects

Healthy controls consisted of two groups: subjects presenting for a routine checkup and patients with positive fecal occult blood screening for whom colon cancer screening colonoscopy was performed without significant

findings. All controls had no prior history of disease and no GI chief compliant.

Biopsy Samples

Colonoscopies were performed by experienced endoscopists. Two specimens from each sample site, rectum and sigmoid, were taken using endoscopic forceps (FB240U Olympus, Tokyo, Japan). The biopsy samples were immediately frozen with liquid nitrogen and stored at −80 °C until use.

Laser-Captured Microdissection (LCM)

The frozen samples obtained at endoscopy were embedded in an optimal cutting temperature compound (Sakura Finetek USA, Inc., Torrance, CA, USA) and cut into serial 8-μm sections. Before microdissection, up to eight sections from each block were mounted on slides and were stained using HistGene LCM Frozen Section Staining Kit (Arcturus Bioscience, Mountain View, CA, USA). Colonic epithelium was isolated using the cryostat sections by a Leica LMD 7000 (Leica Microsystems, Wetzlar, Germany).

RNA Extraction and Quantitative Polymerase Chain Reaction

Total RNA was extracted from whole biopsy samples using RNeasy Mini kit (Qiagen, Hilden, Germany) and from isolated colonic epithelium by LCM using a RNeasy Micro kit (Qiagen), and cDNA synthesis were performed using Super Script III First Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Quantitative reverse transcription (RT)-PCR analysis of *S-100A* and *β-actin* mRNA expression was performed using the StepOnePlus Real Time PCR System (Applied Biosystems, Foster City, CA, USA) employing TaqMan gene expression assay according to the manufacture's instruction (Applied Biosystem). Real-time PCR was performed with cDNA for both target genes and the endogenous control using TaqMan Universal PCR Master Mix (Applied Biosystems). Each amplification reaction was performed in triplicate and the average of the threshold cycles was used. The amount of target was obtained by normalization to an endogenous reference (β-actin) and relative to a calibrator.

Genotyping

Genomic DNA was extracted from 200 μL of EDTA blood using FavoPrep Blood Genomic DNA Mini kits (FAVORGEN, Taiwan). For polymorphism of four 5HTT polymorphisms including the insertion/deletion polymorphism in 5-HTT-LPR and VNTR in intron 2,PCR reactions, PCR-restriction fragment length polymorphism (RFLP) or direct sequencing were performed as described previously using the primers and restriction enzymes in Table 1 [14, 41]. The samples for direct sequencing run on an Applied Biosystems 3130*xl* Genetic Analyzer (Applied Biosystems) following the manufacturer's recommendations.

Questionnaire

Clinical symptoms were assessed by self-rating depression scale (SDS) and gastrointestinal symptom rating scale (GSRS). SDS is a 20-item self-report questionnaire. Each item is scored on a Likert scale ranging from 1 to 4. A total score ranges from 20 to 80. Most people with depression score between 50 and 69, while a score of 70 and above indicates severe depression [41, 42].

The severity of various GI symptoms was assessed using the GI symptom rating scale (GSRS), which is a validated, self-administered questionnaire [42, 43]. Subjects filled out the GSRS questionnaire anonymously. GSRS includes 15 questions on a scale of 1–7, depending on how inconvenient it had been during the previous week. A higher score indicates more inconvenient symptoms. Combination

Table 1 Sequences of primers and restriction enzymes used for genotyping

Genes	Primer sequence (5′–3′)	Restriction enzyme
5-HTTLPR	Forward: 5′-GCCGCTCTGAATGCCAGCAC-3′	
del(s)/ins(l)	Revers: 5′-GGAGGAACTGACCCCTGAAAACTG-3′	
5-HTT-VNTR	Forward: 5′-GTCAGTATCACAGGCTGCGAG-3′	
10rep/12rep	Revers: 5′-TGTTCCTAGTCTTACGCCAGT-3′	
5-HTT	Forward: 5′-CGTAGGAGAGAACAGGGATGCTA-3‘	
G769T	Revers: 5′-CAGCAGCACATGGATTAGAAGGT-3	
5-HTT	Forward: 5′-TTGCCAGGAATTCAGGACT-3′	
C867T	Revers: 5′-TTAAGTGAGAGGAAAGTGGCAG-3′	
IL10		
−1082 G/A	Forward: 5′-CCAAGACAACACTACTAAGGCTTCTTGAGGA-3′	−1082, BseR
−819 C/T	Revers: 5′-AGGTAGTGCTCACCATGACC-3′	−819, MslI

scores among 15 questions can assess the following five domains: reflux symptom (heartburn and acid regurgitation), abdominal pain (stomach ache, gastric hunger pains and nausea), indigestion symptom (gastric borborygmus, gastric bloating, eructation and increased flatus), diarrhea symptom (diarrhea, loose stools and urgent need to defecate) and constipation symptom (constipation, hard stools and feeling of incomplete evacuation).

Analyses

Values are expressed as the mean ± SD or the median with a 25–75 % range, whichever was appropriate depending on whether the data were normally distributed. Statistical analyses for significant differences of parameters were performed using analysis of variance (ANOVA), chi-square analysis, and Kruskal–Wallis test for among the three groups. All pairwise multiple comparison procedures (Bonferroni correction method) among the three groups were performed to isolate the groups from the others. Spearman's correlation coefficient was calculated to examine the correlation. Differences in the genotype frequencies between the two groups and Hardy–Weinberg equilibrium of allele frequencies at individual loci by comparing the observed and expected genotype frequencies were assessed using the chi-square test or the Fisher's exact probability test. The odds ratio (OR) and 95 % confidence interval (CI) were obtained by Mantel–Haenszel statistics. A two-sided p value of less than 0.05 was considered statistically significant. All statistical computations were performed using SPSS (version 11.0 for Windows, SPSS Inc, Chicago, IL, USA).

Results

A total of 156 patients were enrolled including 50 patients with IBS-D, 56 patients with UC, and 50 age- and sex-matched healthy controls. Demographic data and GSRS and scores are shown in Table 2. With the exception of reflux and total GSRS score, the rank order of scores was the IBS group, the UC group and then the controls (Table 2). Scores for indigestion, diarrhea, and constipation were significantly greater in the IBS group than the UC group. SDS scores in the IBS group were significantly greater than in the controls (42 vs. 35, $p = 0.001$) but not between the UC group and the controls (Table 2).

Expression of S100A mRNA

Median expression of S100A8 mRNA in the sigmoid mucosa of UC was significantly greater than in IBS (22×10^{-5} vs. 3×10^{-5}, $p = 0.021$) or controls (22×10^{-5} vs. 3×10^{-5}, $p = 0.015$). Expression in the rectal mucosa of UC was also significantly greater than in the controls (65×10^{-5} vs. 7×10^{-5}, $p = 0.005$). Median expression of S100A9 mRNA in the sigmoid mucosa of UC was significantly greater than in IBS (109×10^{-5} vs. 27×10^{-5}, $p = 0.014$) or controls (109×10^{-5} vs. 27×10^{-5}, $p = 0.006$), and in the rectal mucosa of UC it was also significantly greater than in the controls (250×10^{-5} vs. 25×10^{-5}, $p = 0.009$). The expression of S100A8 and S100A9 mRNA in rectal epithelium isolated by LCM tended to be higher in UC patients than the other groups but the difference was not significant (S100A8 $p = 0.19$, S100A9 $p = 0.32$) (Fig. 1a, b).

S100A10 expression in the rectum in IBS-D was significantly greater (0.121 vs. 0.093, $p < 0.001$) than in UC,

Table 2 Comparison of demographic data and questionnaire scores

Characteristic	Controls, $n = 50$	IBS-D, $n = 56$	UC, $n = 50$	p
Age mean (SD)	41.6 (15.8)	43.1 (16.9)	41.0 (15.3)	0.79[a]
Gender male (%)	34 (68)	39 (69.6)	31 (62)	0.69[b]
GSRS scores				
Reflux	1 (1–1.5)	1.5 (1–2.5)	1 (1–2)	0.005[c]
Abdominal pain	1 (1–1.3)	1.7 (1.3–2.6)	1.3 (1–2.2)	<0.001[c]
Indigestion	1.3 (1–1.5)	2.3 (1.3–3.5)	1.8 (1.3–2)	<0.001[c]
Diarrhea	1 (1–1.7)	3.7 (2.7–5.7)	2.7 (1.7–4.3)	<0.001[c]
Constipation	1.3 (1–2)	2.7 (1.7–3.3)	1.7 (1.3–2.3)	<0.001[c]
SDS scores	35 (31–41)	42 (37–49)	38 (31–45)	<0.001[c]

Values of questionnaire scores are medians and the inter-quartile range (25–75 %)

IBS-D diarrhea predominant irritable bowel syndrome, *UC* ulcerative colitis, *GSRS* gastrointestinal symptom rating scale, *SDS* self-rating depression scale

[a] p values calculated using the analysis of variance (ANOVA)

[b] p values calculated using the chi-square analysis

[c] p values calculated using the Kruskal–Wallis test

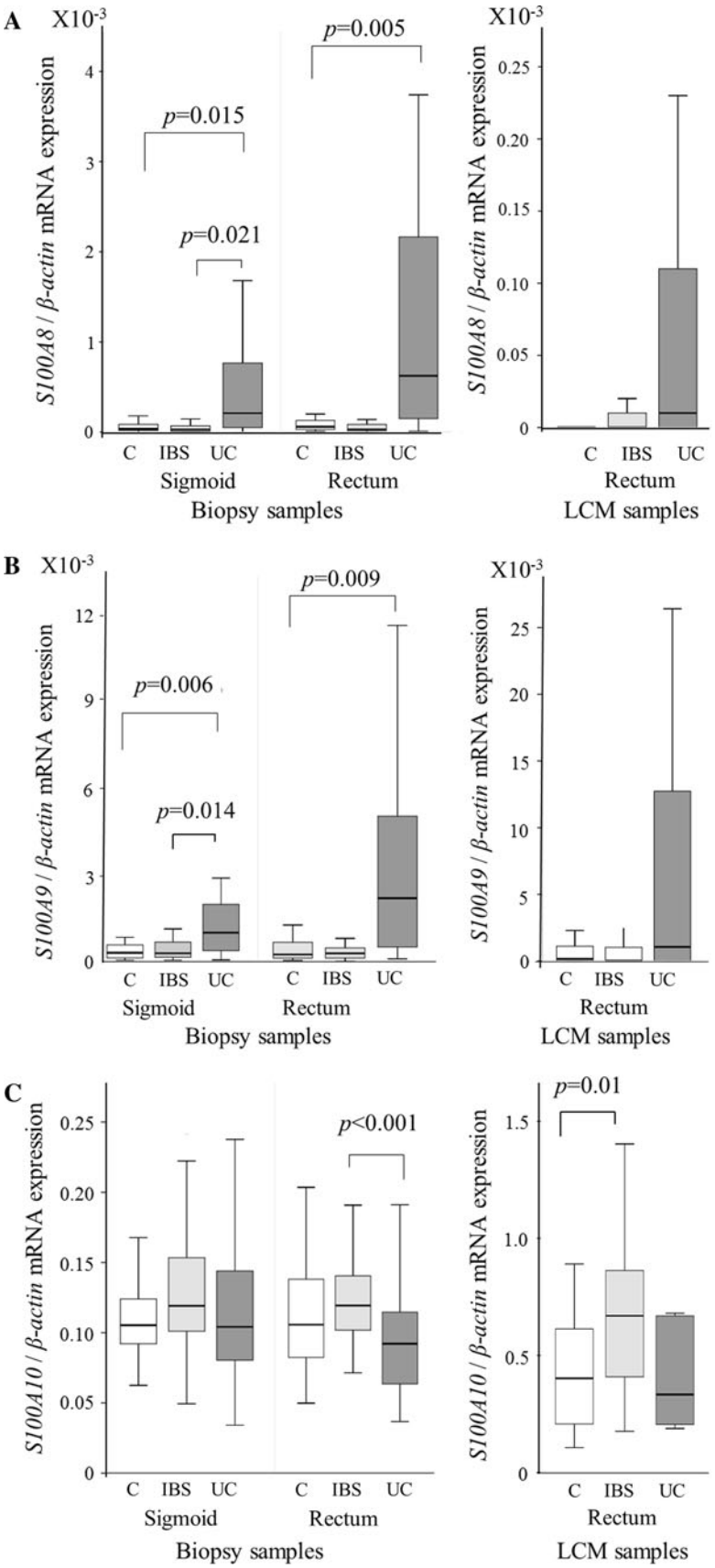

Fig. 1 Comparisons of relative expressions of the S100A mRNA in the colonic mucosa taken by biopsy and rectal epithelium isolated by laser-captured microdissection (LCM) among the controls (C), the diarrhea predominant irritable bowel syndrome group (IBS) and the ulcerative colitis group (UC). *Horizontal bar* median; *box* 25th–75th interquartile range, *vertical lines* range of values. The cycle passing threshold (Ct) was recorded for each mRNA, and *β-actin* was used as the endogenous control for data normalization. *p* values were calculated using Bonferroni correction method between the two groups

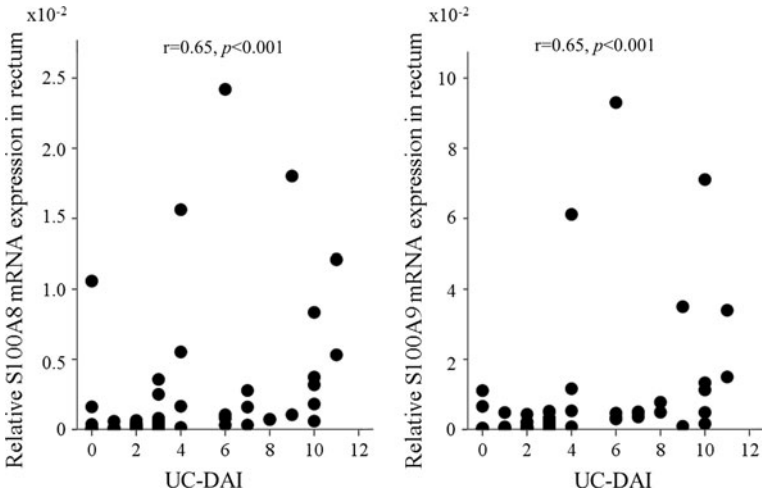

Fig. 2 Correlation between relative expressions of S100A8 or A9 mRNA in the rectum of the patients with ulcerative colitis and the disease activities assessed according to the Sutherland DAI score (UC-DAI). The cycle passing threshold (Ct) was recorded for each mRNA, and *β-actin* was used as the endogenous control for data normalization. *p* values by Spearman's correlation coefficient

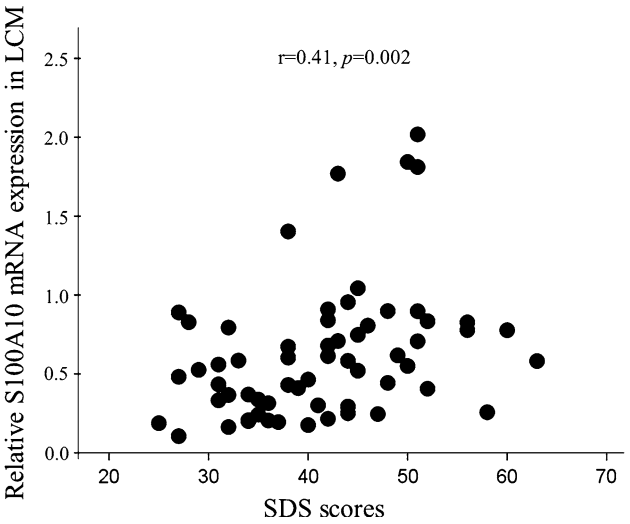

Fig. 3 Correlation between relative expressions of S100A10 mRNA in the rectal epithelium selected by laser-captured microdissection (LCM) and self-rating depression scale (SDS) scores. The cycle passing threshold (Ct) was recorded for each mRNA, and *β-actin* was used as the endogenous control for data normalization. *p* values by Spearman's correlation coefficient

but not in the sigmoid (Fig. 1c). However, the expression of S100A10 in rectal epithelium isolated by LCM was significantly higher (0.669 vs. 0.402, $p = 0.017$) than in the controls (Fig. 1c).

Association of S100A Expression and Clinical Symptoms

S100A8 and S100A9 levels correlated with UC disease activity index ($r = 0.65$, $p < 0.001$) (Fig. 2). S100A10 levels correlated with SDS scores ($r = 0.42$, $p = 0.001$) (Fig. 3), but not with GSRS scores (Table 3).

5HTT and IL10 Genotypes

All 156 subjects were successfully genotyped and the genotypes and allele frequencies are shown in Table 4. The allele frequencies of the polymorphisms did not deviate significantly from those expected under the Hardy–Weinberg equilibrium. The investigated variants of 5HTT genes were not significantly associated with either IBS-D or UC (Table 4).

Table 3 Correlation of S100A mRNA levels with GSRS scores and SDS scores

Scoring	S100A10 Rectal mucosa	S100A10 LCM samples	S100A9 Rectal mucosa	S100A9 LCM samples
GSRS scores				
Abdominal pain	0.14	0.29*	0.14	−0.10
Indigestion	0.25*	0.16	0.25	0.03
Diarrhea	0.05	0.09	0.05	0.02
Constipation	0.09	0.08	0.09	0.10
SDS scores	0.08	0.41**	−0.13	0.06

GSRS gastrointestinal symptom rating scale, *SDS* self-rating depression scale, *LCM* laser-captured microdissection

* $p < 0.05$, ** $p < 0.01$ and *p* values by Spearman's correlation coefficient

Table 4 Allele and genotype frequencies

Variable	Allele frequencies p^a for HWE	Genotype	Control, $n = 50$	IBS-D, $n = 56$	p^b	UC, $n = 50$	p^c
5-HTT	$l = 0.02$	l/l	2	2	0.79	3	0.90
LPR	$s = 0.98$	l/s	13	18		13	
del(s)/ins(l)	$p = 0.99$	s/s	35	36		34	
5-HTT	10rep = 0.12	10/10	1	2	0.99	1	0.82
VNTR	12rep = 0.88	10/12	11	11		8	
10rep/12rep	$p = 0.23$	12/12	38	43		41	
5-HTT	G = 0.91	G/G	32	38	0.75	31	0.21
G769T	T = 0.09	G/T	17	16		14	
	$p = 0.80$	T/T	1	2		5	
5-HTT	C = 0.30	C/C	43	46	0.37	42	0.85
C867T	T = 0.70	C/T	6	10		7	
	$p = 0.95$	T/T	1	0		1	
IL10-819	C = 0.30	C/C	0	6	0.047	8	0.007
C/T	T = 0.70	C/T	24	23		19	
	$p = 0.87$	T/T	26	27		23	
IL10-1082	G = 0.05	G/G	0	0	0.27	0	0.029
G/A	A = 0.95	G/A	2	6		9	
	$p = 0.57$	A/A	48	50		41	

p values by using the chi-square test

[a] Hardy–Weinberg equilibrium (HWE) of allele frequencies at individual loci was assessed by comparing the observed and expected genotype frequencies

[b] Comparison between the diarrhea predominant irritable bowel syndrome (IBS-D) group and the controls

[c] Comparison between the ulcerative colitis (UC) group and the controls

The frequencies of the *IL10-819* CC genotype were significantly higher in IBS-D (10.7 vs. 0 %, $p = 0.047$) and UC (16 vs. 0 %, $p = 0.007$) than in controls. No subject had the *IL10-1082 GG* genotype. The frequency of *IL10-1082 G/A* was significantly higher (18 vs. 4 %, $p = 0.029$, OR 5.3, 95 % CI 1.1–25.9) in IBS-D than in controls.

Discussion

To our knowledge this study is the first to demonstrate association between increased S100A10 expression in the rectal epithelium of patients with IBS-D and depression. It is however unclear whether this is causal, because S100A10 expression is reduced in brain tissue from depressed patients [38], and there is no data on S100A10 expression in the GI tract of depressed patients. It will be interesting to examine S100A10 expression in a group of subjects with IBS-D with and without depression. S100A10 overexpression was observed in the rectum and not in the sigmoid thus confirming the previous observation by Camilleri et al. [39]. They reported that S100A10 was overexpressed in rectum of the patients with IBS-D and in the sigmoid of patients with IBS-C compared to healthy controls. However, the physiological significance of increased S100A10 expression in the rectum remains unclear. S100A10 is involved in the trafficking of members of the voltage-gated sodium and potassium channel families as well as transient receptor potential and chloride channels and plays a selective role in enhancing functional expression of the acid sensitive ion channels (ASCIs), which are involved in visceral nociception [44, 45]. Although S100A10 signaling thus may be relevant to the pain component of IBS, S100 A10 expression in the rectal epithelium only correlated with SDS scores. Further studies on S100A10 expression, especially the physiological correlates and the effect on colonic motor and sensory functions, will be required to identify a specific role, if any.

In contrast, S100A8/A9 expressions in UC were significantly higher than in IBS or controls and correlated with the disease activity index. A previous study in pediatric IBD also indicated overexpression of S100A8/A9 in inflamed mucosa and demonstrated that mucosal S100A8/S100A9 were superior to serum S100A8/S100A9 and all standard inflammatory markers such as ESR, CRP, platelets, and serum albumin in distinguishing IBD from non-IBD controls with 91 % sensitivity and 71 % specificity

[33]. Neutrophils are reported to release S100A8/A9 in response to activation and phagocytosis, and the increased S100A8/A9 in IBD appears to be neutrophil derived [46]. The S100A8/S100A9 expression levels tended to be higher in the rectal epithelium isolated by LCM in our UC patients than in the other groups lacking significant difference indicating that expression of these proteins can also be induced in epithelial cells under inflammatory conditions [28, 29]. The lacking significance is probably because mesenchyme containing neutrophils was excluded in the LCM samples.

Defective IL-10 production in intestinal tissue and blood has been reported in IBD patients [47, 48]. IL-10 production is associated with SNPs at positions −1082 G/A and −819 C/T [26]. Although a direct link between the C-819T SNP and IL-10 production has not been established, the −1082 G and −819 C genotype is more common in IL-10 high producers, whereas −1082 A and −819 T is associated with low IL-10 production [26]. A genetic predisposition for low IL-10 production (A/A at −1082) has been reported to be associated with IBD, particularly with UC [49]. Moreover, Gonsalkorale et al. [50] reported that the high producer IL-10 genotype (−1082 G/G) is less prevalent in IBS patients compared to healthy controls. The lower prevalence of the high producer genotype in IBS and UC suggests that an increased production of IL-10 may have some protective role or, conversely, that individuals predisposed to reduced production of this cytokine might be predisposed to develop IBS. However, the results of a relatively large number of published epidemiology studies investigating the relationship between IL-10 gene and IBD susceptibility are inconsistent and such that the conclusions remain controversial.

A recent meta-analysis reported no association between −1082 SNP and UC susceptibility [51]. In a previous study reported by van der Veek et al. [52], neither IL-10-1082 nor-819 genotypes were associated with IBS. However, they demonstrated that the combination of a low producer IL-10 genotype (−1082 A/A) and a high producer TNF-α genotype (−308 A/A or G/A) appeared more prevalent in IBS-D compared to IBS-C or IBS-A (20 vs. 4 % and 3 %, respectively).

The frequencies of IL-10 genotypes in our subjects are consistent with a recent Japanese report [53]. None of our subjects had the *IL10-1082 GG* genotype, and the frequency of the *IL10-819* CC genotype was lower compared to the Western population. The frequency of *IL10-1082 G/A* was higher in UC compared to controls, but not compared with IBS-D. Moreover, the frequencies of the *IL10-819* CC genotype were significantly higher in both UC and IBS-D compared to healthy controls. The discrepancies in the results of these polymorphism studies are possibly due to racial or regional differences, and the small sample size may have limited our capacity to detect differences between the groups.

Previously reported results on the association of 5HTT-LPR have been conflicting. 5HTT-LPR l/s genotype or s/s genotype have been reported to occur with greater frequency in IBS-D than in controls [14], whereas no association has also been reported [15, 17]. The 5HTT-LPR polymorphism differs according to race, and the prevalence of l/l genotype has been consistently reported as less than 6 % in Korea and Japan [54, 55], which contrasts to the Far Eastern, Turkish and US studies showing a frequency of the l/l genotype as greater than 20 % [14, 15]. The variation in the background prevalence of the l/l genotype may influence the statistical power to detect a genotype-related association.

The major limitation of the present study is possible selection bias especially relating to the relatively small number of cases. A large-scale multicenter study may therefore be required to investigate whether *IL10-819* CC might be a useful tool for the diagnosis and prediction of IBS-D and UC. Another limitation is that our investigation was not based on genome wide screening, and other genotypes including TNF-α-308 and other IL-10 SNPs may have association with clinical severity of IBS-D or UC [52].

In summary, overexpression of S100A10 in the rectal epithelium of IBS patients may be related to its ability to modulate the function of serotonergic receptors including 5-HT1B receptors. The presence of the *IL10-819* CC is a candidate genotype for both IBS and UC in a Japanese population and might be a useful tool for prediction of risk to develop IBS-D or UC.

Conflict of interest None.

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